INTERSTATE QUARANTINE REGULATIONS TO PREVENT THE SPREAD OF PLAGUE IN THE UNITED STATES.

[Department Circular No. 73.—Marine-Hospital Service.]

TREASURY DEPARTMENT,
OFFICE OF THE SECRETARY,
Washington, D. C., May 22, 1900.

To medical officers of the Marine-Hospital Service, State, and local health authorities, and others concerned:

In accordance with the provisions of the act of March 27, 1890, the following regulations, additional to existing interstate quarantine regulations, are hereby promulgated to prevent the introduction of plague into any one State or Territory or the District of Columbia from another State or Territory or the District of Columbia:

1. During the existence of plague at any point in the United States the Surgeon-General of the Marine-Hospital Service is authorized to forbid the sale or donation of transportation by common carrier to Asiatics or other races particularly liable to the disease.

2. No common carrier shall accept for transportation any person suffering with plague or any article infected therewith, nor shall common carriers accept for transportation any class of persons who may be designated by the Surgeon-General of the Marine Hospital Service as being likely to convey the risk of plague contagion to other communities, and said common carriers shall be subject to inspection.

3. The body of any person who has died of plague shall not be transported except in hermetically sealed coffins and by consent of the local health office, in addition to the local representative of the Marine-Hospital Service. Wherever possible, such bodies should be cremated.

L. J. GAGE, *Secretary.*

A MICROBE PATHOGENIC FOR RATS (MUS DECUMANUS AND MUS RATUS) AND ITS APPLICATION TO THE DESTRUCTION OF THESE ANIMALS.—BY J. DANYSZ.

[Translated from Annals of the Pasteur Institute, April, 1900.—By P. A. Surg. H. D. GEDDINGS, U. S. M. H. S.]

Since Lœffler made known his discovery of the bacillus typhi murium, which he isolated from a spontaneous epidemic among white mice, and which he applied with success to the destruction of harvest mice (*M. arvicola*), several other bacteriologists have observed similar epidemics and have isolated the microbes thereof, morphologically identical with the bacillus of Lœffler, but more or less virulent for the various genera and species of the little rodents.

The B. typhi murium was only frankly pathogenic for mice (*M. musculus*) and for harvest mice (*M. arvicola*). The bacillus of Laser was pathogenic for the *M. agrarius;* that of Merechkowski for the *Spermophiles* and finally that of Issatchenko for *white rats.*

Moreover each of the various bacilli is of very variable virulence, so that their practical utilization for the destruction of all species of rodents which they may be brought to bear upon has been fraught with many difficulties.

It would evidently be of great interest, first, to extend the field of action of one of them by increasing its virulence and thus rendering it capable of attacking other species of rodents; then, this virulence increased, to be able to maintain it at its highest point. I have endeavored to solve this problem and the following is what I have arrived at:

A cocco-bacillus presenting all the characteristics of the B. coli, and in this resembling the bacillus of Lœffler, isolated by me from a spontaneous epidemic among harvest mice, has shown itself from the beginning as slightly pathogenic for gray rats (*M. decumanus*). Out of 10 animals fed with a culture of this microbe, 2 or 3 would die; several others would sicken, but would recover; others still appeared completely refractory.

The fact that a certain number of animals fed with these cultures always succumbed permitted the hope that it would be possible to increase the virulence of the microbe by generally employed methods, that is to say, by a certain number of passages from rat to rat.

A great number of experiments executed to this end always showed that successive passages from rat to rat, whether by feeding or by subcutaneous injection, ended by enfeebling rather than by increasing the virulence of the microbe given by ingestion. We proved always that if the culture, in a first passage, killed animals in seven to twelve days, and if the second and third passages were shown to be a little more virulent and killed in five to ten days, the cultures from succeeding passages became less and less virulent and always ended by not killing at all.

It was rarely possible to go beyond 10 to 12 passages. Sometimes the series was stopped at the fifth passage, or even sooner, by the survival of all the animals undergoing experiment. The result was exactly the same if instead of alternating each passage through the animal by a culture in bouillon or on agar, the bodies of animals dead of a preceding passage were fed to others.

It was, therefore, certain that in the evolution of an epidemic caused by the microbe it was necessary to take account, in order to explain its extinction, not only of the natural resistance of the survivors, but of an indisputable diminution of the virulence of the microbe.

The following experiment gives a direct proof of it:

A lot of 30 normal mice were placed in a cage with 2 sick mice; another lot of 30 mice were placed in 6 different jars and fed with the same culture as the sick mice placed in the cage with the 30 normal ones. In the 6 jars all the mice were dead in from four to six days; in the cage an epidemic broke out in three days after the death of the 2 first sick mice, whose bodies were devoured. This epidemic lasted

twenty-three days; 27 mice died, three survived the experiment but succumbed later in consequence of the ingestion of a culture of average virulence.

These 3 mice then were neither completely refractory nor immunized, and their resistance in the first experiment can only be explained by the attenuation of the virulence of the microbe in the cage.

As this microbe is but slightly toxic and only kills after having passed from the intestines into the economy, where it ends by increasing enormously, it appeared to me to be indicated to seek the principal cause of the attenuation of virulence in the change of media which the microbe experienced successively in the digestive tract and in the blood, and to which it had to become habituated successively in passing from one animal to another.

In fact we note in a very constant manner that on the one hand an increase of virulence for the blood and organs obtained by a long series of subcutaneous injections, coincides with a notable diminution of virulence by the digestive tube; on the other hand we demonstrate in an equally regular manner, that microbes isolated from the blood or spleen of an animal at the period when they commence to pass from the intestine into the blood, are always found to be more virulent by ingestion than those which are isolated after the death of the animal, that is to say, after a more or less long cultivation in the juices of the economy.

Finally it is to be noted that passages of cultures in collodion sacks, inclosed in the peritoneal cavity of rats, whether in uninterrupted series, or by alternating each sack culture with culture in bouillon or on agar, end invariably by a notable diminution of virulence administered by the digestive tract.

Increase of virulence and its preservation.

The foregoing observations inspiring me, and having proved that a virus which killed mice in four to six days, commenced to pass from the intestine to the blood, and was accumulated in the spleen in the twenty-four hours after the ingestion, I succeeded in increasing its virulence, and rendered it regularly pathogenic for rats by following the method here described.

A bouillon culture, isolated from the blood twenty-four hours after the ingestion of a virus which was mortal in four to five days, was left for twenty-four hours in the incubator and was replanted in new bouillon and distributed in flasks, as completely filled as possible. The flasks were placed first in the incubator until the culture developed, and then kept at ordinary temperatures, until a deposit formed and the bouillon became perfectly clear. This may take four or five days, and its object is to accustom the microbe to an anærobic existence. From the flasks we pass the culture in a collodion sack which is kept for twenty-four to thirty-six hours in the abdominal cavity of a rat, then it is planted anew in ordinary bouillon, and thence again into flasks. The

culture from these last flasks is then planted on agar, and it is these cultures on agar which we give to mice to eat, after having diluted them with water, and soaked bread or grain in the dilution.

This series of operations is repeated several times, and at the fourth or fifth repetition we note a very decided increase of virulence. Mice which only died at the end of four to seven days, now die in thirty-six to sixty hours after the ingestion.

When we have obtained such a result we may replace the mice by white rats, commencing with young rats, a month or six weeks old, and as we continue the passages we may take older rats. Proceeding thus, and making sack cultures in the abdominal cavities of the species of animals which we desire to infect, we may specialize the culture, and may render it sufficiently virulent in ten passages.

Operating in this manner I have succeeded in rendering regularly virulent at first for gray rats (*M. decumanus*), and then for black rats (*M. ratus*), and finally for white rats, a culture which was originally but slightly virulent for the gray rat, and entirely innocuous for the other two.

The bouillon which I used was a bouillon of horse meat, with 1 per cent peptone, and to which was added a little carbonate of lime to neutralize the acids which are formed during culture, and which rapidly diminish the virulence of the microbe.

Contained in the flasks and kept from the influence of light and air, the cultures preserve their virulence for several months. Planted on agar, they preserve it without appreciable diminution for two months; in bouillon in flasks or tubes, stoppered with cotton, they alter very rapidly.

These are the cultures, relatively stable, which I have tried to use for the destruction of rats in sewers and in other localities which they infest. As I have said above, there are two factors in the development of an epidemic among animals: (1) a pathogenic microbe, and (2) the species of animal in which it is desired to produce it. We know that the various species of rats do not resemble each other. We have seen, too, that the properties of the same species of rats are not everywhere the same, and depend in a certain measure upon the conditions of their feeding. The question was to know what proportion of success or failure could proceed from all these badly studied causes of variation; for this, experiment was necessary.

Practical application—Results obtained.

Cultures brought, little by little, to a degree of virulence, permitting the killing by ingestion of all rats placed in cages in the laboratory, in from five to twelve days, have been used in a great number of experiments on farms, stores, and other localities infested by rats. The sum of the reports, figuring several hundred which I have received, shows that in 50 cases out of 100 there was a total disappearance of rats; in 20 cases

the results appeared entirely negative; in 30 others there was an appreciable diminution of rats noted.

In certain quite rare cases we have been enabled to follow the extension of an epidemic from a treated locality to one not treated.

Observations of this kind are interesting but scarcely permit us to precisely appreciate the real effects of the intervention. The number of sick rats and cadavers which are truly found is always very small, and it is impossible to know certainly whether the rats which have disappeared have succumbed to the disease or have simply emigrated, fleeing from the epidemic.

Thus, when the sanitary service of Paris asked of the Pasteur Institute if it were possible to destroy the rats in the sewers by means of a contagious disease, I deemed it necessary, before answering, to submit this question to a special study.

I asked of the chief engineer, M. Bechmann, and of the inspectors of sewers, Mm. Masson and Delphini, to have placed at my disposal a sewer trunk closed on all sides to prevent the escape of rats, abundantly furnished with straw and food, and to introduce therein a fixed number of living, healthy rats from neighboring sewers.

These conditions having been complied with in a sewer 160 meters long and 3 meters wide, the experiment gave the following results:

On February 2, 200 gray rats (*M. decumanus*) were released in the sewer and kept under observation for ten days. On the 12th, the sewer was carefully visited and all the rats appeared well; not a single cadaver was found. On the same day 20 tubes of culture on small pieces of bread were distributed in the sewer. The epidemic began on February 20, and a second distribution of virulent culture was made. Until March 2 the sewer was visited daily, and 80 cadavers of rats were found, of which 40 were necropsied and the others left in place. Those necropsied showed without exception the characteristics of the disease (congestion of the intestines, hypertrophy of the spleen, etc.), and contained pure cultures in the blood. The rats left in place were always eaten from one day to the next by the survivors.

March 2 we could not discover, with the most careful search, anything but a quantity of shapeless débris, not permitting an estimate of the number of rats devoured, and 8 live rats which were permitted to escape by the negligence of the watchman.

Though the experiment was thus not followed to an end, it shows in a definite fashion that the rats at liberty in the sewers always eat freely the bread soaked in culture bouillon, in spite of an abundance of other food (wheat, carrots, etc.), that they contract the disease and die in large numbers, and that the survivors eat the bodies.

It is therefore very possible to create by the aid of this culture, epidemics, which then to a certain extent, propagate themselves.

The spread of the epidemic will probably be quite limited, as it will be stopped after the third or fourth passage by attenuation of the viru-

lence, always experienced in our studies as related above and also in consequence of the greater resistance of a certain number of the survivors. Thus, when it is desired to destroy a large majority of the rats which infest a locality, the culture must be distributed at intervals of ten to twelve days, that is to say, at the period when the preceding distribution will have produced its effect.

The season of the year in which this treatment ought to be applied is not altogether a matter of indifference. The young rats are much more susceptible to the action of the virus than the old ones, and the epidemics will be more deadly in spring (April, May, and June) and in autumn (September to December) than at other periods of the year.

By systematically destroying during ten successive years, the young generations, which succumb inevitably, we would finish by destroying all rats in a most complete manner.

Experiments made simultaneously at Lille, by M. Calmette, director of the Pasteur Institute of Lille; at Hamburg by Dr. Abel, sanitary physician; at Copenhagen by M. Th. Madsen; and at Tunis by M. Loir, director of the bacteriological station, have given very nearly the same results.

Rats in a cage have always succumbed after an ingestion of culture, in from eight to twelve days, the greater number of wholesale experiments resulting in a total or very complete disappearance of rats.

THE CONSTITUENTS OF HAFFKINE'S ANTIPLAGUE VACCINE.—BY S. S. MALLANNAH, M. B.

[Extracted from the British Medical Journal, May 12, 1900.]

I have carried out several experiments in order to find out the real immunizing constituent of Haffkine's prophylactic. The prophylactic fluid was passed through a Pasteur-Chamberland filter.

(1) The sediment found on the bougie consisted of bodies of dead plague bacilli. It was proteid in reaction and protected rabbits from plague in doses of 300 mg.

(2) The filtrate, a clear fluid, also gave proteid reactions and possessed well-marked protective power, even in small doses. It is possible to isolate, after Brieger's method of preparing tetanus toxin, the immunizing substance from Haffkine's fluid in a more or less pure condition, and this immunizing substance does not respond to any of the known proteid reactions, and possesses well-marked protective power in rabbits against plague in doses of 100 mg. This immunizing substance is gray and amorphous, and is soluble in water. It might be termed extracellular, as it is found dissolved in the surrounding media. The immunizing substance present in the sediment might be called intracellular, as it is found in the bodies of the dead bacilli.

The extracellular immunizing substance is found in the bodies of the cells (plague bacilli), and is then thrown into the surrounding media,